3.6 IDENTIFICATION OF HCMV-PP65 DERIVED CYTOTOXIC T CELL EPITOPES AS POTENTIAL SYNTHETIC VACCINES

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The $CD8^+$ class I restricted response to HCMV plays a crucial role in the control of HCMV infection in asymptomatic immunocompetent hosts. Many attempts have been undertaken to induce protective cytotoxic T cell (CTL) response *in vivo* in immunocompromised pati nts. The observation that proteins entering the cytoplasm after HCMV fusion are sufficient to stimulate a CTL response has directed attention to the major matrix protein pp65, one of the protein constituents of the capsid of the HCMV virion. In order to identify CTL epitopes for their possible use as peptide-vaccine candidates, the HCMV-pp65 protein sequence was screened and a set of 17 peptides which fulfil the binding motif for HLA-A*0201 molecules were synthesised. Such peptides were tested, both for their ability to stabilise the HLA-A*0201 molecules on the surface of the mutant cell line T2 and by a peptide competition assay. These assays showed that 6 of the 17 peptides were able to bind effectively to HLA class I molecules at the cell surface. All of these peptides had the secondary anchor residues known to be associated with strong binding to HLA-A*0201 alleles. To evaluate the involvement in natural HCMV infection, the strongly binding peptides were used in the stimulation of T cell lines to assess their capacity to generate a peptide specific response *in vitro*. Three of those peptides, 2 from the amino terminal and one fom the carboxiy terminal region stimulated CTL responses in HCMV seropositive individuals *in vitro*. Peptide-induced CTL's recognised the immunising peptide loaded on T2 cells as well as CMV infected HLA-A*0201 fibroblasts. These CTL's were capable of recognising not only the synthetic peptide but also the naturally processed pp65 in an HLA-A*0201 restricted manner. The identification of this pp65 immunogeneic epitopes may prove useful in the development of potential peptide vaccines which will help to amplify a memory CTL response to HCMV. Alternatively the strategy proposed here for the generation of autologous CTL's specific for pp65 could be used in adoptive T cell immuneotherapy for the selective reconstitution of CMV-specific immunity in bone marrow transplant recipients.

Keywords CMV CTL peptide vaccines

4.1 HUMAN ANTIGEN PRESENTING CELL / TUMOUR CELL HYBRIDS AS CANDIDATE CANCER VACCINES

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Most tumours do not stimulate effective anti-tumour immune responses *in vivo*. In order to enhance the immunogenicity of human tumour cells, we have fused a variety of tumour cell lines with an Epstein-Barr virus transformed B lymphoblastoid cell line (EBV B-LCL) *in vitro*, to produce stable hybrid cells. Hybrid cell lines showed a marked increase in their ability to stimulate primary allogeneic T cell responses *in vitro*, as compared with the parent tumour cells. Both CD4+ and CD8+ T cell responses were induced. The stimulatory hybrids expressed HLA class I and class II, and a wide range of surface accessory molecules including the T cell costimulatory ligand molecules CD40, CD80 (B7.1) and CD86 (B7.2). The interaction of CD80 and CD86, and to a lesser extent CD40, with their surface receptors on the responding T cells was required for optimal stimulation of T cell responses. Fusion of the EBV B-LCL with a melanoma cell line (518.A2) yielded hybrid c lls which presented the melanoma antigens MAGE-1 and MAGE-3 to antigen-specific, HLA class I restricted cytotoxic T lymphocyte (CTL) clones with greater efficiency than the parent melanoma cell line. These findings indicate that the generation of human antigen presenting cell / tumour cell hybrids offers considerable promise as an approach to cancer immunotherapy.

Keywords cancer vaccine cell hybrids
antigen presentation

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